azitra

NYSE: AZTR

Cutaneous delivery of LEKTI via an engineered strain of Staphylococcus epidermidis for the treatment of Netherton syndrome

Travis Whitfill MPH MPhil PhD(c)

Society of Investigative Dermatology Annual Meeting

Precision dermatology powered by synthetic biology.

FORWARD-LOOKING STATEMENTS

This document contains forward-looking statements concerning Azitra, Inc. (“Azitra”, the “Company,” “we,” “us,” and “our”). The words “believe,” “may,” “will,” “potentially,” “estimate,” “continue,” “anticipate,” “intend,” “could,” “would,” “project,” “plan,” “expect” and similar expressions that convey uncertainty of future events or outcomes are intended to identify forward- looking statements. These forward-looking statements include, but are not limited to, statements concerning the following:

- our future financial and operating results;
- our intentions, expectations and beliefs regarding anticipated growth, market penetration and trends in our business;
- the timing and success of our plan of commercialization;
- our ability to successfully develop and clinically test our product candidates.

These forward-looking statements are subject to a number of risks, uncertainties and assumptions, including (i) we are an early-stage clinical biopharmaceutical company with limited operating history, (ii) there are no drug products to date that incorporate our microbial library and genetic engineering platform and the clinical and commercial utility of our microbial library and genetic engineering platform is uncertain and may never be realized; (iii) we have only recently commenced Phase 1 clinical studies of our initial product candidates and our product candidates will require extensive additional preclinical and clinical testing; (iv) we expect we will need additional financing to execute our business plan and fund operations, which additional financing may not be available on reasonable terms or at all; and (v) those other risk described in “Risk Factors” section of the prospectus (“Prospectus”) dated June 15, 2023 filed by Azitra with the Securities and Exchange Commission on June 21, 2023.

In light of these risks, uncertainties and assumptions, the forward-looking events and circumstances discussed in this document may not occur and actual results could differ materially and adversely from those anticipated or implied in our forward-looking statements. You should not rely upon forward-looking statements as predictions of future events. Although we believe that the expectations reflected in our forward-looking statements are reasonable, we cannot guarantee that the future results, levels of activity, performance or events and circumstances described in the forward-looking statements will be achieved or occur. Azitra does not undertake and specifically disclaims any obligation to update or revise our forward-looking statements to reflect new circumstances or unanticipated events as they occur, except as required by law.

Personal disclosures

Employment/compensation

Current

- Azitra Inc. (NYSE: AZTR) (Cofounder, COO)*
- Actuate Therapeutics Inc. (Cofounder)*
- Inspired Spaces LLC (Cofounder)*
- LetsImproveHealth LLC (Founder)*
- Umbrex LLC (Healthcare management consultant)*
- Yale University (Assistant Professor Adjunct)

Former

- Bios Partners LP (Former Partner)*
- Bios Research (Former Senior Analyst)
- Cue Biopharma (NASDAQ: CUE) (Former consultant)
- Encore Vision (Former consultant)
- Novartis (Former consultant)
- TFF Pharma (NASDSQ: TFFP) (Former consultant)

Shareholder

- Azitra Inc. (NYSE: AZTR)*
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- i-Lumen Scientific*
- Immusoft Corporation*
- IN8bio (NASDAQ: INAB)*
- Lantern Pharma (NASDAQ: LTRN)*
- Aileron Therapeutics (NASDAQ: ALRN)*
- ONL Therapeutics*
- Opus Genetics*
- SIRPant Immunotherapeutics (Former board member)*
- Stream Biomedical*
- Trefoil Therapeutics*

Current Board of Directors

For-profit

- Azitra Inc. (NYSE: AZTR)*
- IN8bio (NASDAQ: INAB)*

Non-profit (No financial interest)

- International Network for Simulation-based Pediatric Innovation, Research, and Education (INSPIRE) (Treasurer)
- International Pediatric Simulation Society (IPSS) (Treasurer)

Grant/research support (PI)

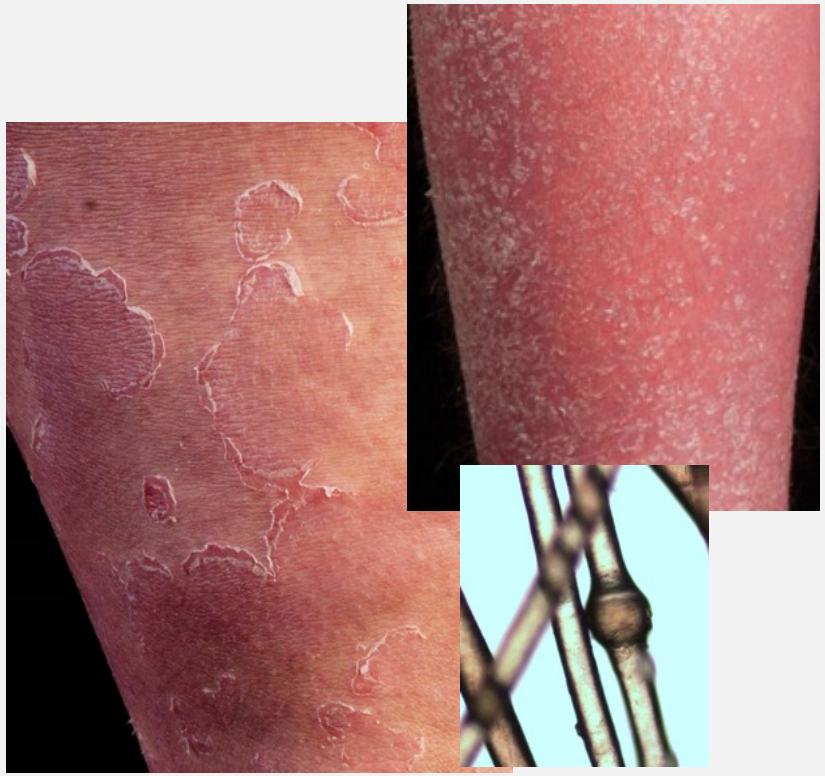
- Connecticut Innovations
- Connecticut Biosciences
- Defense Advanced Research Projects Agency (DARPA)
- Department of Defense (DoD)
- Indiana University
- Macy Foundation
- National Institutes of Health – NIAMS
- National Institutes of Health – NIAID
- National Science Foundation (NSF)
- Thiel Foundation
- Yale University

***Current financial interest**

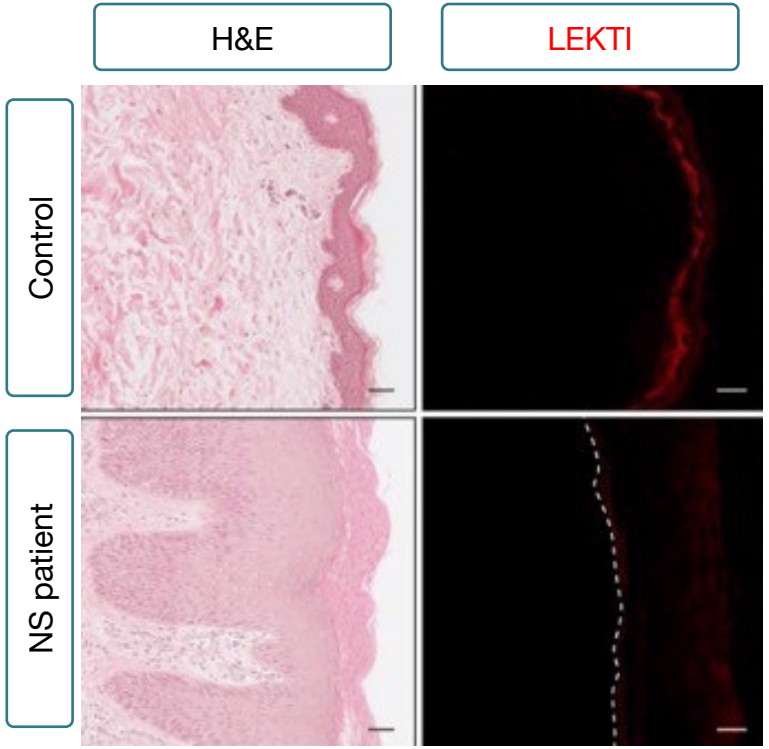
Netherton Syndrome

- **Netherton syndrome (NS)** is a rare, autosomal recessive disease
  - Estimated to occur in 1:100,000- 1:200,000 live births
  - Global prevalence of ~20,000
- Classic clinical triad of congenital ichthyosiform erythroderma, trichorrhexis invaginate (‘bamboo hair”), and an atopic diathesis
  - Caused by mutations in the serine protease inhibitor of Kazal type 5 (*SPINK5)* gene, which encodes the serine protease inhibitor, LEKTI (lympho-epithelial Kazal-type related inhibitor)
  - Loss-of-function mutations in *SPINK5* lead to unregulated kallikrein (KLK) proteolysis and activation of the KLK cascade
  - Overactive proteases cause desquamation and scaling, skin barrier defects, increased transepidermal water loss, pruritus
  - Microbial and allergen penetration trigger release of antimicrobial peptides and activation of inflammatory pathways, including IL-36γ
  - Multisystem complications, including life-threatening dehydration, failure to thrive, and sepsis in infancy with ~10% mortality rate
  - No approved therapies for NS

Clinical Presentation

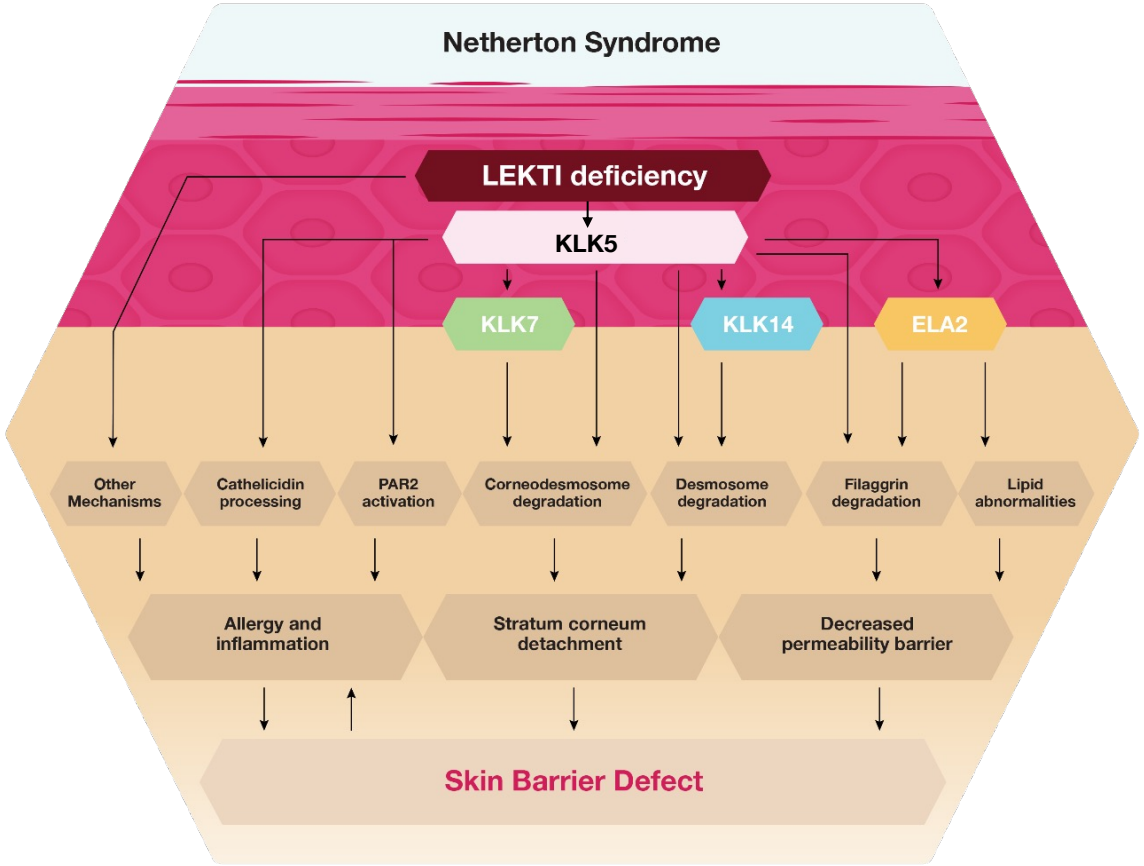


LEKTI is Deficient in Skin Affected by Netherton Syndrome



Mintoff, Fischer, *Mol Genet Genomic Med.*, **2021**, *9*, e1611

Targeting KLK5 in Netherton Syndrome via Expression of LEKTI



- LEKTI fragments inhibit KLK5, KLK7 and KLK14, which are involved in desquamation, PAR-2 activation, degradation of lipid hydrolases
- Uncontrolled serine protease activity leads to defects in skin barrier, and release of pro-inflammatory and pro-allergic mediators, causing the disease manifestations of Netherton syndrome
  - Activation of inflammatory pathways is associated with elevation of IL-36γ

ATR-12: LEKTI-Secreting *Staphylococcus epidermidis*

- **ATR-12 is a live biotherapeutic product,** comprised of an **auxotrophic strain** of *Staphylococcus epidermidis* (SE351) engineered to express domain 6 (D6) of the human LEKTI protein
  - ATR-12 is formulated as a topical ointment (ATR12-351)
- **Mechanism of action:** Delivery of recombinant human LEKTI-D6 (rhLEKTI) in the lower layers of the stratum corneum restores KLK activity, while the *S. epidermidis* strain reduces IL-36γ. Auxotrophic ATR-12 inhibits the overactive proteases through LEKTI fragment secretion.
  - Inhibit skin serine protease activity, improve skin barrier function, reduce underlying cutaneous inflammation, and improve the cutaneous signs and symptoms (appearance, pain, and pruritus) of Netherton syndrome

Key Facts

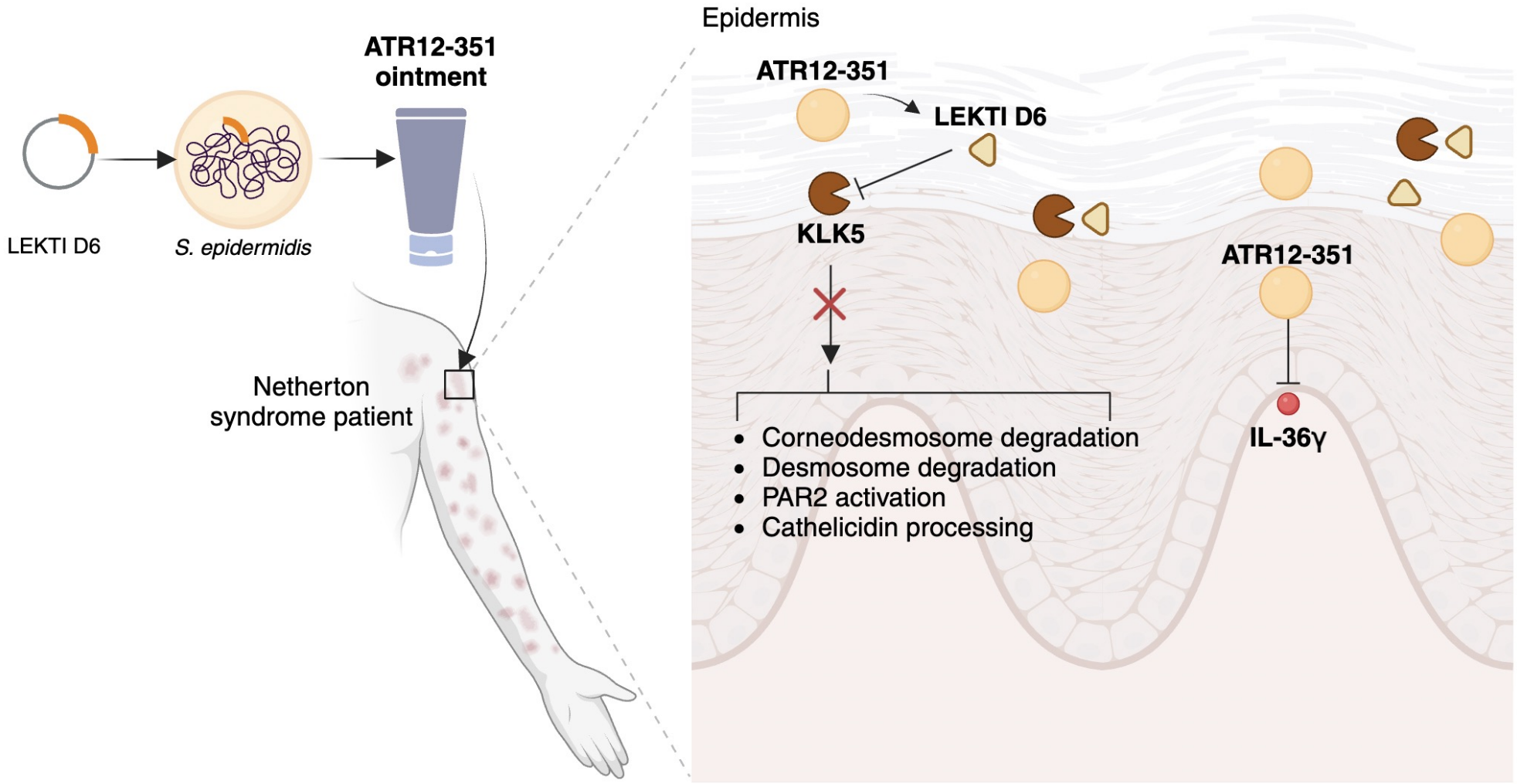
Primary Mechanism:
Restore kallikrein levels

Pharmacologic Effects (on HSE):
Reduction of trypsin-like activity (protease activity) by 7-fold

Topical Formulation:
Effectively and substantially delivers LEKTI throughout skin

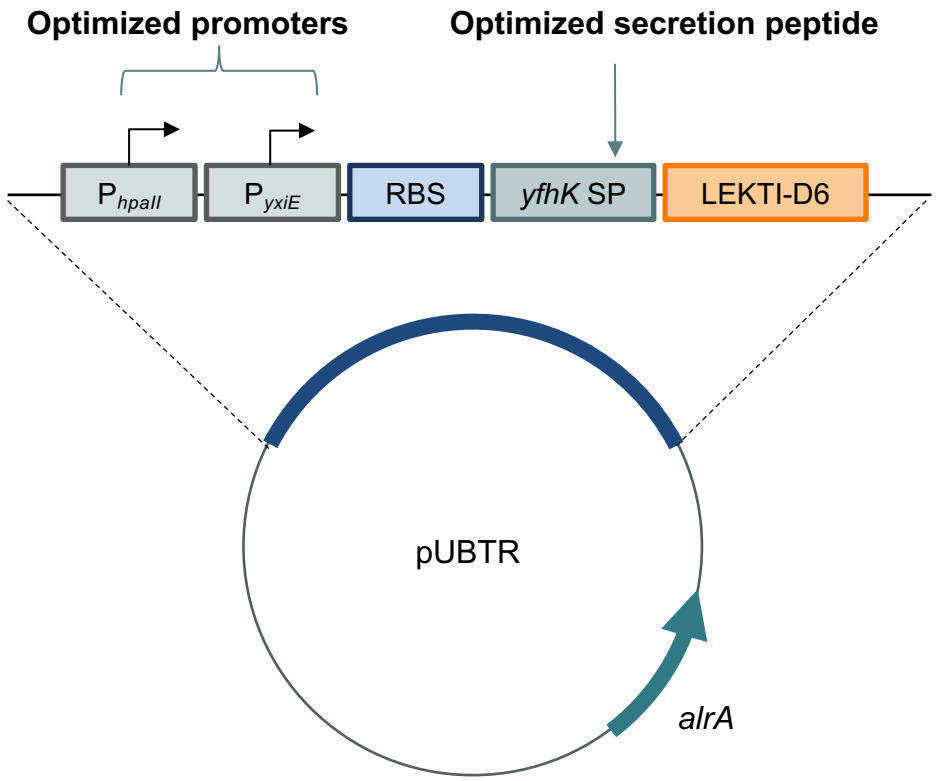
Clinical Development Status:
Phase 1b

Mechanism of Action of ATR12-351 to Treat Netherton Syndrome

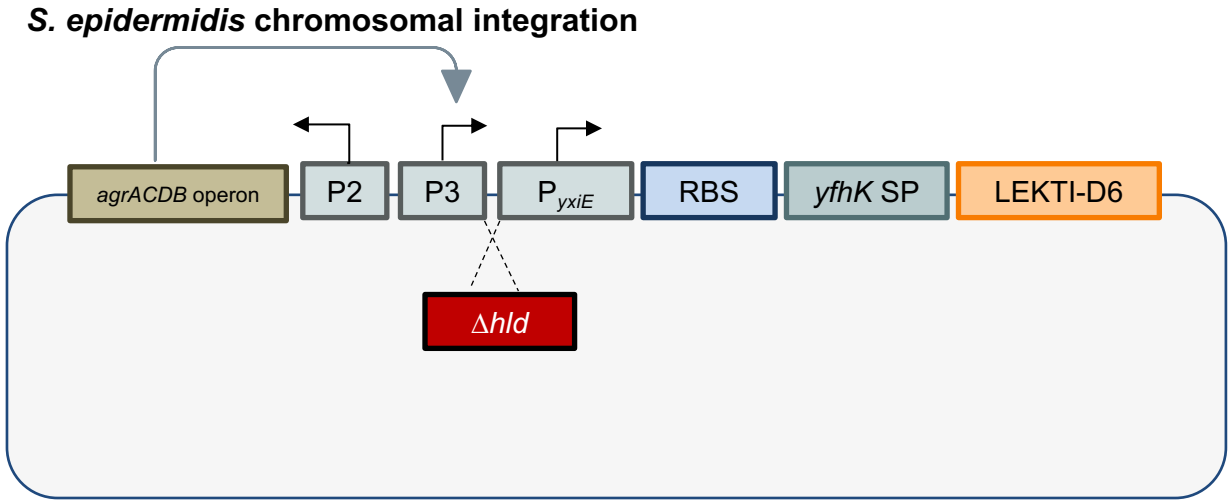


Design of ATR12-351: an auxotrophic, LEKTI-D6 secreting strain of *S. epidermidis*

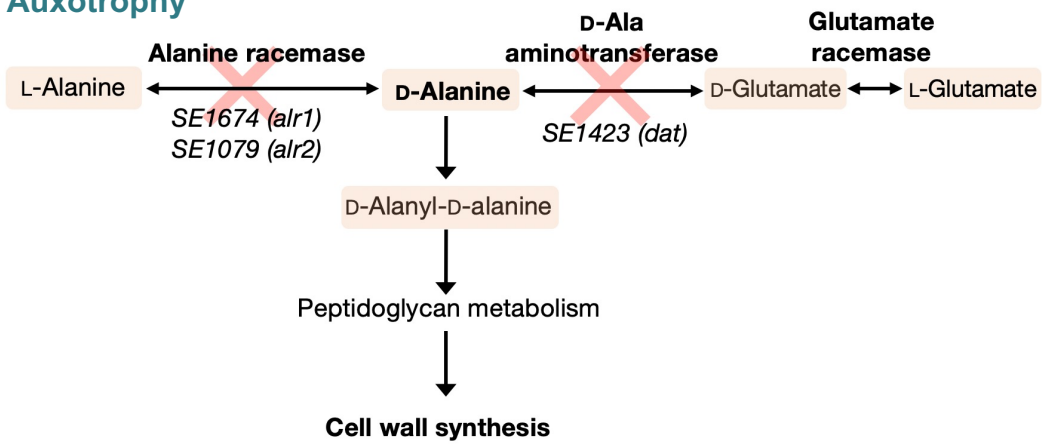
SE27a: Plasmid expression of LEKTI-D6



SE351: Chromosomal integration of LEKTI-D6 and D-alanine auxotrophy

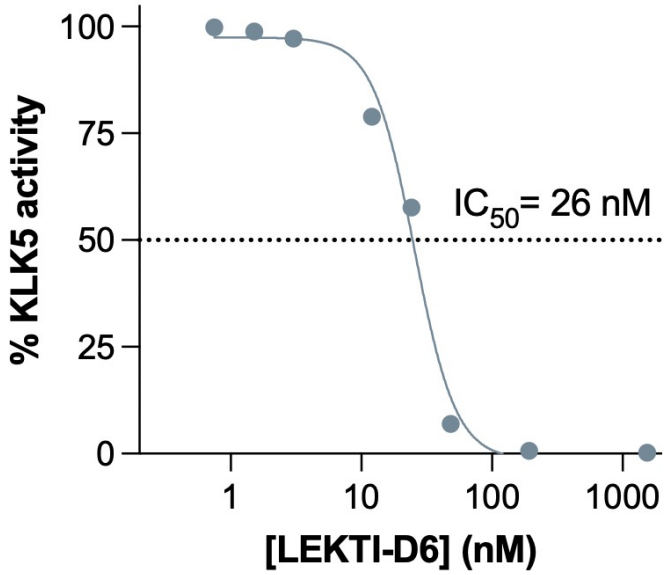


Auxotrophy

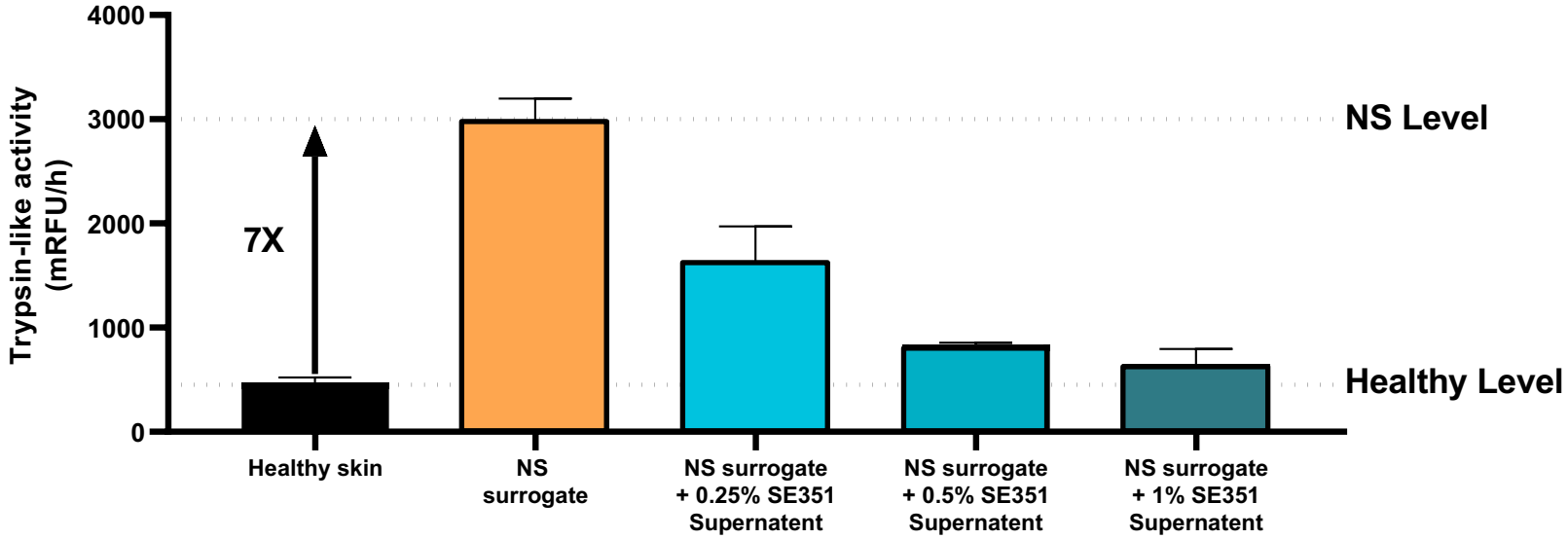


Confirmation of SE351 Potency and Activity

Nanomolar IC50 of KLK5 from SE351 *in vitro*



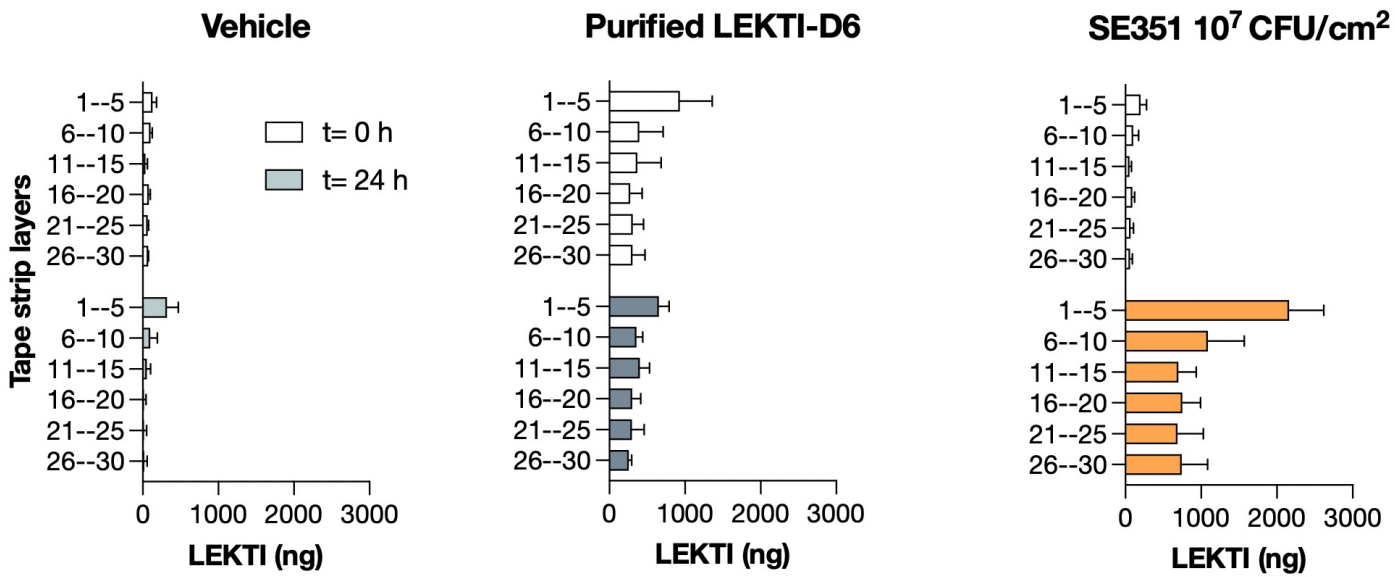
***S. epidermidis* strain SE351 (LEKTI-secreting) spent broth inhibition of human skin tape stripped extracts**



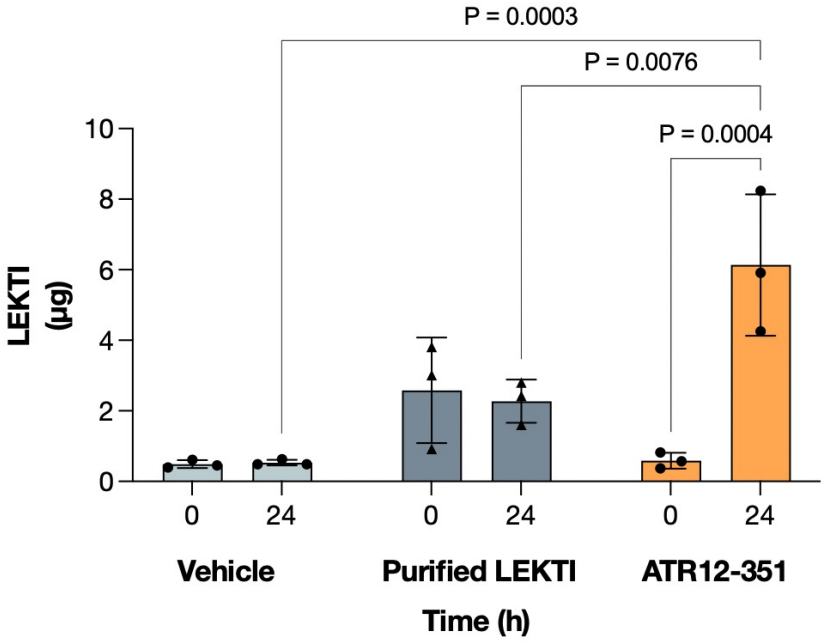
- Trypsin-like activity (key measure of protease activity) decreased after addition of spent broth from LEKTI-secreting strain SE351
- Dose-dependent response across concentrations of supernatant

ATR-12 Provides Superior LEKTI Delivery Compared to Topical LEKTI Delivery in *ex vivo* Full Thickness Human Skin

LEKTI by skin layer



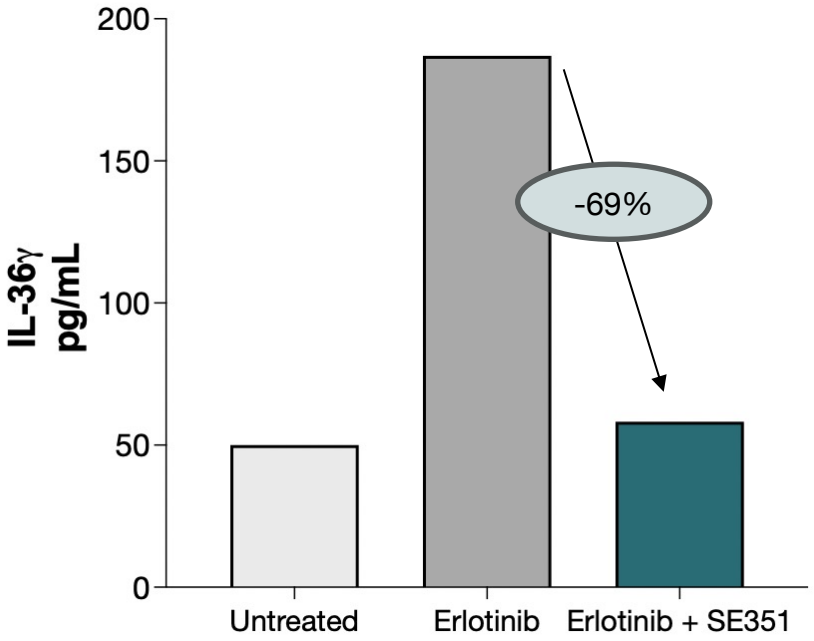
Total LEKTI amounts



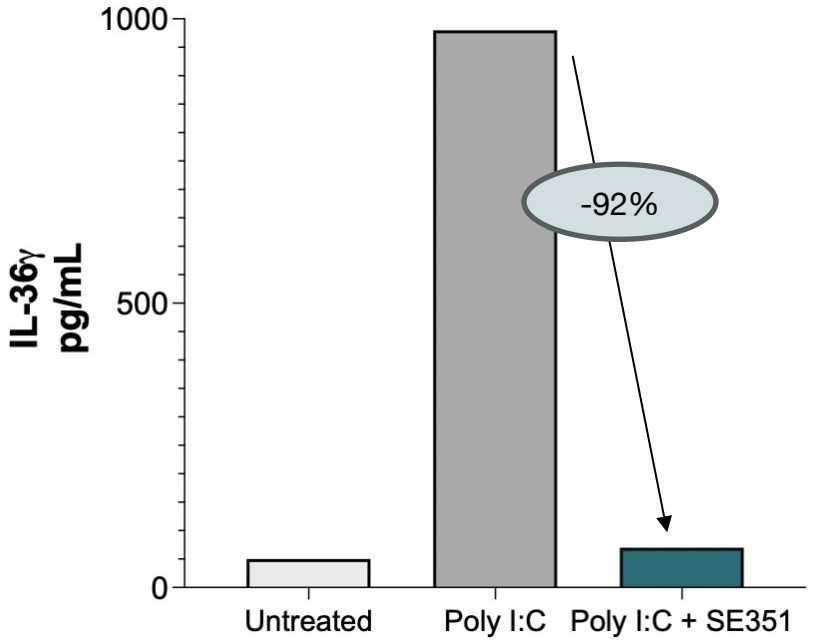
- ✓ LEKTI activity is significantly higher after 24 hours compared to vehicle and topical protein alone in all layers following ATR-12 application
- ✓ The LEKTI activity penetrates to at least 30 layers deep in substantial amounts

SE351 Potently Reduces IL-36γ

Reconstructed epidermis induced with erlotinib and treated with SE351 cells

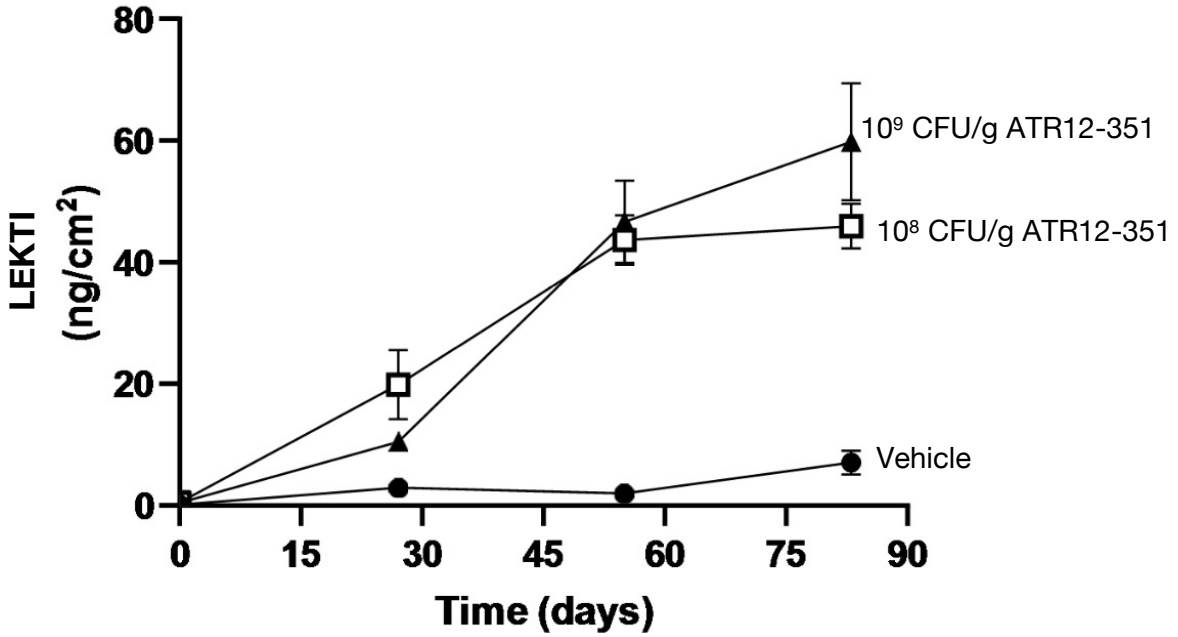


HaCaT cells induced with Poly I:C and treated with SE351 cell-free supernatant



ATR12-351 Delivers High Amounts of LEKTI in Minipigs and is Safe and Well Tolerated

LEKTI delivery by ATR12-351 over time



- ATR12-351 delivers high amount LEKTI to skin of minipigs through 90 days
- ATR12-351 was safe and well-tolerated in minipigs with abraded skin and SE351 was not detected in the blood

Summary

- ATR12-351, a LEKTI-expressing strain of S. epidermidis in development for Netherton syndrome (NS) has demonstrated key proof of concept in preclinical studies:
  - **Nanomolar IC_{50} values** to inhibit KLK5, a key driver of NS
  - Delivers functional LEKTI and **reduces protease activity** to normal levels in NS models with *ex vivo* human skin
  - Delivers LEKTI **significantly more effectively than LEKTI delivery alone**
  - **Reduces IL-36γ** in HaCaT cells and reconstructed human epidermis
  - **Delivers high amounts of LEKTI** over 90 days in minipigs and is well-tolerated
- Azitra has an open Phase 1b clinical trial in NS (NCT06137157)

Acknowledgements

Azitra, Inc.
David Dodds PhD
Trudy Grossman PhD
Alaina Kaiser PhD
Roger Leger PhD
Dorival Martins PhD
Sara Mootien PhD
Azim Munivar MD
Marie-Aude Pinoteau PhD
Maryam Rajaee PhD
Francisco Salva
Mark Sampson PhD
Mary Spellman MD
Alex Tikhonov PhD

Genoskin
Kimbria Blake PhD

Jackson Laboratory
Julia Oh PhD Lab

Paraza Inc.
Peter White PhD
David Charboneau PhD
Majida El Bakkouri PhD
Steven Huynh

Yale University
Leonard Milstone MD

Funding sources:
CT Innovations
CT Biosciences New Haven
National Science Foundation (NSF) #1853071
National Science Foundation (NSF) #1648819
National Institute of Health (NIH) / NIAMS R43AR073562
National Institute of Health (NIH) / NIAID 1R41AI130373-01
Thiel Foundation BL-01537
Yale University Center for Engineering, Innovation, and Design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THANK YOU
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